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Effects of flow scarcity on leaf-litter processing under oceanic climate conditions in calcareous streams

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HIGHLIGHTS

- The effects of droughts on litter decomposition in temperate streams were assessed.
- The contribution of decomposers and detritivores was studied.
- Detritivore activity is more affected than microbial decomposition by droughts.
- Water properties condition the effects of droughts on macroinvertebrate behavior.

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ABSTRACT

Although temporary streams represent a high proportion of the total number and length of running waters, historically the study of intermittent streams has received less attention than that of perennial ones. The goal of the present study was to assess the effects of flow cessation on litter decomposition in calcareous streams under oceanic climate conditions. For this, leaf litter of alder was incubated in four streams (S1, S2, S3 and S4) with different flow regimes (S3 and S4 with zero-flow periods) from northern Spain. To distinguish the relative importance and contribution of decomposers and detritivores, fine- and coarse-mesh litter bags were used. We determined processing rates, leaf-C, -N and -P concentrations, invertebrate colonization in coarse bags and benthic invertebrates. Decomposition rates in fine bags were similar among streams. In coarse bags, only one of the intermittent streams, S4, showed a lower rate than that in the other ones as a consequence of lower invertebrate colonization. The material incubated in fine bags presented higher leaf-N and -P concentrations than those in the coarse ones, except in S4, pointing out that the decomposition in this stream was driven mainly by microorganisms. Benthic macroinvertebrate and shredder density and biomass were lower in intermittent streams than those in perennial ones. However, the bags in S3 presented a greater amount of total macroinvertebrates and shredders comparing with the benthos. The most suitable explanation is that the fauna find a food substrate in bags less affected by calcite precipitation, which is common in the streambed at this site. Decomposition rate in coarse bags was positively related to associated shredder biomass. Thus, droughts in streams under oceanic climate conditions affect mainly the macroinvertebrate detritivore activity, although macroinvertebrates may show distinct behavior imposed by the physicochemical properties of water, mainly travertine precipitation, which can override the flow intermittence effects.

1. Introduction

Temporary rivers and streams represent a high proportion of the total number, length and discharge of running waters around the world (Larned et al., 2010; Acuña et al., 2014). Nevertheless, in spite of their numerical importance, historically the study of intermittent streams has received less attention than that of perennial ones (Datry et al., 2011a). Drought events can be imposed by natural and anthropogenic factors, such as climatic conditions, lithological features of watersheds (e.g. karstic geology) or damming and water withdrawals. Nowadays, related with predictions of global climate change on temperature and rainfall dynamics (IPCC, 2013), the number of ecological studies on temporary streams is growing exponentially (Datry et al., 2011a), specially in semi-arid and Mediterranean regions (e.g., Muñoz, 2003; Bonada et al., 2006; Álvarez and Pardo, 2009; Sponseller et al., 2010), which suffer a drought period during summer. Moreover, these works are mainly focused on biotic communities and structural attributes, but the consequences of desiccation on ecosystem processes are still poorly studied.

A key process in the functioning of low-order forest streams is leaf litter decomposition, since detritus inputs from surrounding vegetation

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constitutes the main source of matter and energy in these systems (Wallace et al., 1997). Leaf litter decomposition is influenced by a series of environmental factors such as temperature (Ferreira and Chauvet, 2011; Martínez et al., 2014), catchment land use (Lecerf and Richardson, 2010; Martínez et al., 2013a), dissolved nutrients (Greenwood et al., 2007; Pérez et al., 2012), pH (Larrañaga et al., 2010; Dangles et al., 2004) or oxygen saturation (Medeiros et al., 2009). One of the main drivers of this process in streams is flow regime, leaf processing being consistently lower in temporary streams than in perennial systems due to the lack of aquatic detritivores as well in semi-arid regions (Herbst and Reice, 1982; Boulton, 1991; Maamri et al., 1997; Pinna and Basset, 2004) as under oceanic climate conditions (Langhans and Tockner, 2006; Datry et al., 2011b; Schlief and Mutz, 2011). However, the effects of droughts on microbial activity vary between regions, being less severe under oceanic conditions since the humid weather during winter can maintain substrates moist enough for microbial development (Langhans and Tockner, 2006).

Despite regular and abundant rainfalls, in oceanic climate regions, natural intermittent streams are often found in calcareous areas, even during fall-winter, the rainiest period. Chalk streams are especially susceptible to prolonged drought (Berrie, 1992) due to the porosity of calcareous substratum. Waters of calcareous streams present high concentrations of $CaCO_3$. In fact, in some calcareous streams a travertine precipitation may occur resulting in a layer that covers the stream bottom and the standing substrates. This feature influences litter decomposition, enhancing the process when the travertine deposition is not continuous (Carter and Marks, 2007; Miliša et al., 2010) or slowing down when a compact and continuous layer happens (Casas and Gessner, 1999). In this kind of systems the effects of droughts have been studied, but mainly focused on structural attributes as fauna (Agnew et al., 2000; Boulton, 2003), lacking information about the effects on a functional attribute as leaf litter decomposition.

Thus, our main objective was to assess the effects of flow cessation on litter decomposition under oceanic climate conditions in calcareous streams. For this, leaf litter of alder (*Alnus glutinosa* L. Gartner), a common and autochthonous tree species in riparian forests of oceanic climate regions in Europe, was incubated in four calcareous streams with different flow regimes. The relative importance and contribution of decomposers and detritivores to litter processing as a function of flow scarcity were evaluated. We hypothesize that 1) drought events negatively affect leaf litter decomposition and 2) the effects of droughts are more pronounced on the process mediated by detritivores than that of mediated by microorganisms.

2. Materials and methods

2.1. Study site

The study was carried out in four calcareous streams (S1, S2, S3 and S4) with different flow regimes in the headwater of the Nervión River catchment (Basque Country, north Spain). One of the streams (S3) presented a continuous travertine layer covering the bottom. The climate is oceanic with an average annual air temperature of around 14 °C and a mean annual precipitation around 1000–1200 mm. The four streams drain catchments of more than one hundred hectares (Table 1), a usual threshold for a permanent discharge regime in this region for siliceous streams (among the so many streams studied by us in the region for the last two decades, only the work by Otermin et al. (2002) has shown flow cessation in a stream which catchment was 83 ha). However, in the present study in calcareous streams, the observed differences in water flow stability are not directly related with the size of the catchment area, but probably the geologic substrate and its distribution in the basin. All catchments are covered mainly by natural vegetation dominated by forests of Portuguese oak *Quercus faginea* Lam.

2.2. Stream discharge and drought characterization

During the study period (24 November 2011–24 April 2012), we measured stream discharge biweekly to monthly (Martin Marten Z30, Current-meter) and recorded qualitative information about the flow condition (running water, disconnected pools, dry channel) to assess conditions of drought. Additionally, we collected stream discharge data at 10-minute intervals from a gauging station (www.bizkaia.net) located 7–10 km downstream in the main course of the same catchment (43° 2′ 4″N, 2° 59′ 59″ W), from 1 September 2011 to 30 April 2012.

Table 1
Location, reach characterization and water physicochemical characteristics of the studied streams (mean ± SE; n = 6–10). For water temperature, daily mean values and their range are shown (n = 121–154). Days of flow cessation represent the days with absence of superficial flow from 1 September 2011 to 30 April 2012.

	S1	S2	S3	S4
Latitude	42° 58′ 30″ N	43° 1′ 45″ N	42° 59′ 11″ N	43° 0′ 44″ N
Longitude	3° 1′ 43″ W	3° 5′ 22″ W	2° 58′ 13″ W	3° 3′ 58″ W
Basin (km^2)	3.11	3.54	1.54	4.07
Altitude (m a.s.l)	390	520	400	570
Width (m)	5.2 ± 0.6	3.9 ± 1.5	1.40 ± 0.3	4.0 ± 1.6
Land cover (%)				
Rocky area	12.1	24.0	2.4	13.5
Native vegetation	53.6	58.8	86.1	67.3
Forest plantations	11.6	0.0	1.4	0.0
Pasture	22.7	17.2	3.4	19.2
Other	0.0	0.0	6.7	0.0
Flow ($L\ s^{-1}$)	123.2 ± 70.2	49.4 ± 20.9	8.5 ± 3.0	34.8 ± 16.2
Flow cessation (days)	0	0	74	129
Water temperature (°C)	9.10 (6.45–15.02)	6.73 (2.41–11.00)	8.00 (5.81–10.17)	6.80 (3.17–10.65)
% O_2 saturation	100.3 ± 1.2	100.7 ± 1.5	103.5 ± 3.2	108.0 ± 3.6
Conductivity (μS/cm)	353.6 ± 12.9	289.0 ± 26.2	450.1 ± 34.0	290.0 ± 19.5
Alkalinity ($meq\ L^{-1}$)	2.97 ± 0.10	2.61 ± 0.21	3.75 ± 0.24	2.68 ± 0.19
pH	8.07 ± 0.11	8.35 ± 0.16	8.17 ± 0.18	8.53 ± 0.14
Chloride ($mg\ Cl\ L^{-1}$)	10.43 ± 0.97	5.13 ± 1.15	19.08 ± 1.07	5.63 ± 0.82
Sulfate ($mg\ S\ L^{-1}$)	3.18 ± 0.32	1.98 ± 0.22	5.21 ± 0.22	1.91 ± 0.40
Calcium ($mg\ Ca\ L^{-1}$)	39.4 ± 1.6	31.8 ± 1.7	47.5 ± 1.8	33.9 ± 1.7
Sodium ($mg\ Na\ L^{-1}$)	6.20 ± 0.71	3.05 ± 0.20	12.25 ± 0.44	3.16 ± 0.44
SRP ($\mu g\ P\ L^{-1}$)	12.00 ± 3.53	11.68 ± 2.46	10.98 ± 1.98	11.23 ± 5.83
Nitrate ($\mu g\ N\ L^{-1}$)	918.7 ± 76.0	438.2 ± 47.3	139.5 ± 13.8	688.2 ± 142.0
Nitrite ($\mu g\ N\ L^{-1}$)	1.95 ± 0.30	1.94 ± 0.30	1.94 ± 0.32	1.93 ± 0.41
Ammonium ($\mu g\ N\ L^{-1}$)	44.7 ± 2.5	42.9 ± 1.9	40.5 ± 4.0	44.3 ± 2.3

In order to define the discharge regime for each stream, the discrete flow measures and visual inspections were compared with the continuum data from the downstream flow monitoring gauging station. According to these observations we determined a downstream discharge threshold for the interruption of the surface water flow at each studied stream. While all the studied streams presented variable discharge regimes (Table 1), only S3 and S4 showed several periods with the absence of superficial flow (6 and 8 drought events respectively representing 74 and 129 days of the 8 months considered).

2.3. Stream water characteristics

During study period, water physicochemical properties were characterized in the four streams on 6–10 occasions. Each time, oxygen saturation, conductivity, pH (WTW multiparametric sensor) and river flow were measured in situ. Additionally, water samples were collected from each stream with polyethylene bottles and transported to the laboratory in refrigerated chambers for alkalinity and nutrient analyses. In laboratory, water samples were immediately filtered (0.7 μm pore size glass fiber filters, Whatman GF/F). Subsamples of the filtered water were used to determine alkalinity by titration to an end pH of 4.5 (APHA, 2005). Nitrate and other ion concentrations were determined by capillary ion electrophoresis (Agilent CE), ammonium was measured by the manual salicylate method, nitrite was measured by the sulphanylamide method and soluble reactive phosphorus (SRP) was measured by the molybdate method (APHA, 2005).

2.4. Leaf litter breakdown

Leaves of common alder *A. glutinosa* (L.) Gaertner were used. In October 2011, freshly fallen leaves were collected from forest soil just after natural abscission. Leaf-litter was air dried, and approximately 3 g (±0.25) was placed separately into fine mesh bags (15 × 20 cm, 0.5 mm mesh size) and 5 g (±0.25) was placed into coarse mesh bags (15 × 20 cm, 5 mm mesh size). In S1 and S2 bags were placed in the streams on the 24th of November 2011. In each site, 4 iron bars were anchored randomly to the streambed along 50 m and 8 bags (4 samples × 2 mesh size) were tied to each bar by nylon lines. In total, 16 bags were placed in each streambed. An additional bar in one of the streams (S1) was used to tie 4 extra bags of each mesh size to determine mass loss by leaching (24 h after immersion). Litter bags were retrieved (4 fine bags and 4 coarse bags from each stream) after 7 days ($t7$) and, thereafter, when losses roughly correspond to 25% ($t25$), 50% ($t50$) and 75% ($t75$) of the initial mass (corrected by leaching). In the other two streams (S3 and S4) the absence of surface flow did not allow us to place the bags in the same date. Thus, the implantation of bags was delayed until the flow recovery, 26th of December 2011, following the same methodology as in the former two sites. In order to discard differences due to this relative delay we decided to start the incubation of a new set of bags in S1 and S2 that we call control bags (4 fine and 4 coarse bags per stream), which were retrieved at the same time than the last sampling date in S3 and S4. In each sampling date, after retrieved, the litter bags were placed individually in plastic bags and transported in refrigerated containers to the laboratory. The leaf material from each bag was rinsed with filtered stream water on a 500 μm sieve to remove sediments and associated macroinvertebrates. Then, it was oven dried (70 °C, 72 h) and weighed to determine leaf dry mass. Subsamples from each bag were ground and stored (−20 °C) for nutrients analyses (see below), and the rest was combusted (500 °C, 4 h) to determine ash free dry mass (AFDM).

2.5. Leaf litter stoichiometry

The carbon and nitrogen concentrations were determined with a Perkin Elmer series II CHNS/O elemental analyzer and phosphorus concentration was determined spectrophotometrically after mixed acid digestion (molybdenum blue method; Allen et al., 1974). Results were expressed as percentage of elemental contents (C, N and P) of leaf litter dry mass.

2.6. Macroinvertebrates

At $t50$ sampling (31 days of incubation in S1 and S2 and 29 days in S3 and S4), macroinvertebrates from bags were sorted out on a 0.5-mm sieve and preserved in 70% ethanol. In the laboratory, macroinvertebrates were identified to family level (Oligochaeta to order, Chironomidae to tribe) using Tachet et al. (2002). All individuals were counted and sorted into shredders and non-shredder macroinvertebrates (after Tachet et al., 2002; Merritt and Cummins, 2007). The dry mass of shredders and of the rest of invertebrates was determined by oven-drying (70 °C; 72 h). For each sample, taxa richness and Shannon diversity index were calculated.

In the same sampling date of $t50$, five benthic samples (Surber 0.09 m^2, 0.5 mm mesh size) were taken from randomly chosen riffles to characterize the macroinvertebrate community of each stream. In the laboratory, the same methodology as for associated fauna was carried out.

2.7. Statistical analyses

Leaf litter breakdown rates (b) were estimated by a linear model ($M_t = M_0 - b \times t$; where M_t is the remaining % AFDM at time t, M_0 is the initial % AFDM after correcting for leaching, and b is the loss rate). The decay rates were compared by two-way ANCOVA (factors: stream, mesh size) with time as covariant. To compare processing rates separately by mesh sizes one-way ANCOVA (factor: stream) was used. As control bags were retrieved in a single sampling date, the leaf mass losses were transformed into rates considering the same model. To compare these processing rates with those reported in the other two streams the same methodology to estimate the rates was used being M_t the remaining mass at time t ($t70$). The comparison among the rates calculated with this method was carried out by one-way ANOVA (factor: stream). Leaf-C, -N, -P and percentage of ash along the incubation were tested by three-way ANOVA (factors: stream, date, mesh size). Differences in benthic and leaf litter associated macroinvertebrates (density and biomass) and water characteristics among the four studied streams were tested using one-way ANOVA (factor: stream). Bivariate relationships were tested by linear regression analyses. When necessary, data were transformed ($\log_{10}(x+1)$). All statistical analyses were performed using R statistical program (version 2.11.1; R Development Core Team, 2012).

3. Results

3.1. Stream water characteristics

All streams presented well oxygenated waters and typical values of calcareous streams such as high conductivity and alkalinity (hard-waters) and pH over 8 (Table 1). However, S3, the travertine stream, showed higher values in conductivity (ANOVA: $F_{3,28} = 9.35$, $p < 0.001$), alkalinity (ANOVA: $F_{3,28} = 7.07$, $p = 0.001$), chloride (ANOVA: $F_{3,28} = 37.85$, $p < 0.001$), sulfate (ANOVA: $F_{3,28} = 27.90$, $p < 0.001$), calcium (ANOVA: $F_{3,28} = 16.30$, $p < 0.001$) and sodium (ANOVA: $F_{3,28} = 67.22$, $p < 0.001$) than the other streams. The phosphorus availability was low in all the streams, being dissolved nitrogen, mainly nitrate, more variable among sites (Table 1; ANOVA: $F_{3,28} = 20.36$, $p < 0.001$). During the incubation period in the intermittent streams, only S4 presented periods of surface flow interruption (during the 3rd and 4th days and during the 11th day after bag establishment).

3.2. Leaf litter breakdown

In fine mesh bags, *b* ranged from 0.39 d^{-1} in S4 to 0.58 d^{-1} in S1, and did not differ statistically among streams (Fig. 1; ANCOVA: $F_{3,49} = 1.93$, $p = 0.137$). Coarse-mesh *b*-values ranged from 0.52 d^{-1} in S4 to 1.64 d^{-1} in S3 and differed among study sites (ANCOVA: $F_{3,54} = 5.37$, $p = 0.003$), *b* being lowest in S4 (Fig. 1). In general, *b* was faster in coarse bags than in fine bags, (ANCOVA: $F_{1,103} = 53.23$, $p < 0.001$). The exception was S4 (ANCOVA: $F_{3,103} = 4.44$, $p = 0.006$), where there were no significant differences in breakdown rate between fine and coarse mesh bags (ANCOVA: $F_{1,24} = 1.33$, $p = 0.261$). When considering the control bags, again there were not differences among streams in fine bags (ANOVA: $F_{3,8} = 2.16$, $p = 0.170$) and in coarse bags S4 showed the lowest value (Fig. 1; ANOVA: $F_{3,8} = 23.39$, $p < 0.001$).

3.3. Leaf litter stoichiometry

After leaching, values of C, N, P and ash in alder leaves were respectively 49.35%, 3.10%, 0.084% and 4.14% (fine mesh bags), and 50.10%, 3.09%, 0.074% and 4.32% (coarse mesh bags). During in-stream incubation the %C differed among streams (ANOVA: $F_{3,85} = 45.52$, $p < 0.001$) and sampling dates (Fig. 2; ANOVA: $F_{3,85} = 50.85$, $p < 0.001$) in the two mesh bag types. In general, leaf-C concentration diminished through time, with greater reduction in S3. Nitrogen concentration varied among streams (ANOVA: $F_{3,85} = 12.65$, $p < 0.001$), sampling dates (ANOVA: $F_{3,85} = 4.61$, $p = 0.005$), as well as between mesh types (Fig. 2; ANOVA: $F_{3,85} = 54.42$, $p < 0.001$). While in fine bags the %N increased along the process, except in the material incubated in S3, in coarse bags the highest values were reached in the middle stages of the incubation. The materials in fine bags reached higher nitrogen concentration than those in coarse bags. However, this pattern did not repeat in all streams (ANOVA: $F_{3,85} = 9.13$, $p < 0.001$). In fact, the material incubated in S4 did not differ in %N among mesh sizes (ANOVA: $F_{1,20} = 3.25$, $p = 0.086$). Leaf-P differed also among streams (ANOVA: $F_{3,85} = 25.36$, $p < 0.001$), sampling dates (ANOVA: $F_{3,85} = 17.32$, $p = 0.001$) and between mesh types (Fig. 2; ANOVA: $F_{1,85} = 44.30$, $p < 0.001$).The leaf-P concentration in S4 tended to remain constant along the time. In the other streams there were differences in leaf-P among dates but without a clear dynamic. Between mesh bags, the material incubated in fine bags presented higher P concentrations than that from coarse bags.

The leaf ash content differed also among streams (ANOVA: $F_{3,85} = 208.25$, $p < 0.001$) and sampling dates (Fig. 2; ANOVA: $F_{3,85} = 289.50$, $p < 0.001$), but not between mesh types. This content increased along incubation time, the material from S3 presenting the highest values, approximately fifteen fold higher at final stage than those at initial one.

3.4. Benthic and associated macroinvertebrates

Forty different taxa of macroinvertebrates were identified from the benthos of all streams (27 in S1, 24 in S2, 15 in S3 and 17 in S4), S3 and S4 presenting five times lower density than S1 (Fig. 3; ANOVA: $F_{3,16} = 8.69$, $p = 0.001$). Among the total number of identified organisms, the shredders represented the 34.6%. Shredder density was also much lower in S3 and S4 than in S1 (Fig. 3; ANOVA: $F_{3,16} = 5.62$, $p = 0.008$). Moreover, the relative importance (percentage) of shredders differed among streams (47.9% in S1, 23.1% in S2, 11.5% in S3 and 8.3% in S4). The specific composition and the dominance of certain species also differed among streams. In S1 the shredder *Echinogammarus* was the most abundant taxon (42.9%), while S3 and S4 were dominated by Oligochaeta (49.2 and 72.5% respectively). Stream S2 showed the most even distributed macroinvertebrate community, with five taxa (*Nemoura*, *Perla*, *Polycelis*, *Habroleptoides* and *Heptagenia*) representing more than 10% each. In terms of biomass, S3 and S4 presented also lower values of total invertebrates (ANOVA: $F_{3,16} = 12.36$, $p < 0.001$) and shredders (ANOVA: $F_{3,16} = 11.31$, $p < 0.001$) than S1 (Fig. 3). As in the case of density, the relative importance of shredder biomass differed among streams (68.6% in S1, 42.3% in S2, 20.6% in S3 and 27.6% in S4).

Of the 40 taxa identified from benthos 33 were found associated with leaf-litter bags (12 in S1, 18 in S2, 17 in S3 and 8 in S4). S2 and S3 presented the highest density of organisms per bag, while S4 presented the lowest one (Fig. 3; ANOVA: $F_{3,12} = 4.69$, $p = 0.022$). Regarding shredder density, the highest value was found in S2 and the lowest one in S4 (Fig. 3; ANOVA: $F_{3,12} = 7.29$, $p = 0.005$). The relative importance of shredders associated with bags was higher in S1 (62.5%) and S2 (71.7%) than in S3 (26.7%) and S4 (15.4%). While in S1 the assemblages colonizing leaf bags reflected the benthic taxonomic distribution and the shredder *Echinogammarus* was the most abundant taxon (37.5%), this pattern did not repeat in the bags incubated in the other three streams. The shredder *Nemoura* represented 50.0% in bags from S2, while the dipteran Scyrtimidae was the dominant taxon associated with leaf bags in S3 and S4. It is remarkable the great number of macroinvertebrates in bags incubated in S3, where benthic samples showed very low densities. In terms of biomass, S2 showed the highest values of total invertebrates and shredders, and S4 showed the lowest ones (Fig. 3). However, these differences did not reach statistical significance ($ANOVA_{Tot}$: $F_{3,12} = 2.40$, $p = 0.118$; $ANOVA_{Sh}$: $F_{3,12} = 3.06$, $p = 0.069$). Moreover, while shredder biomass represented more than 85% of total biomass in S1, S2 and S3, it only attained to 20.5% in S4.

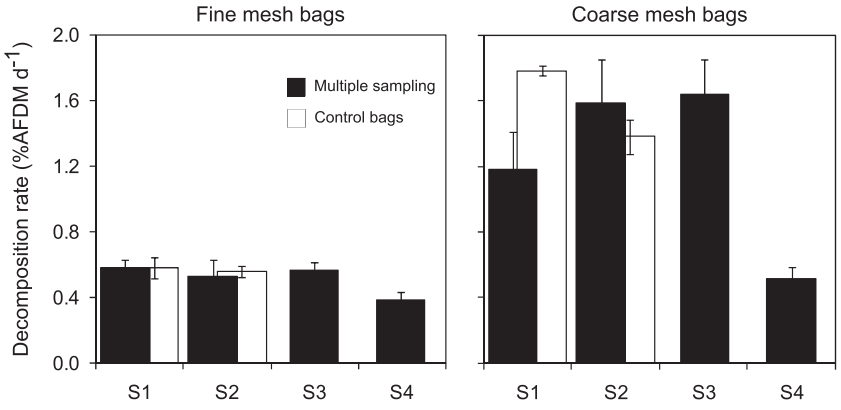

Fig. 1. Decomposition rates (%AFDM d^{-1}; mean ± SE) in fine (left) and coarse (right) mesh bags. Multiple sampling series (black bars) and control bags (white bars).

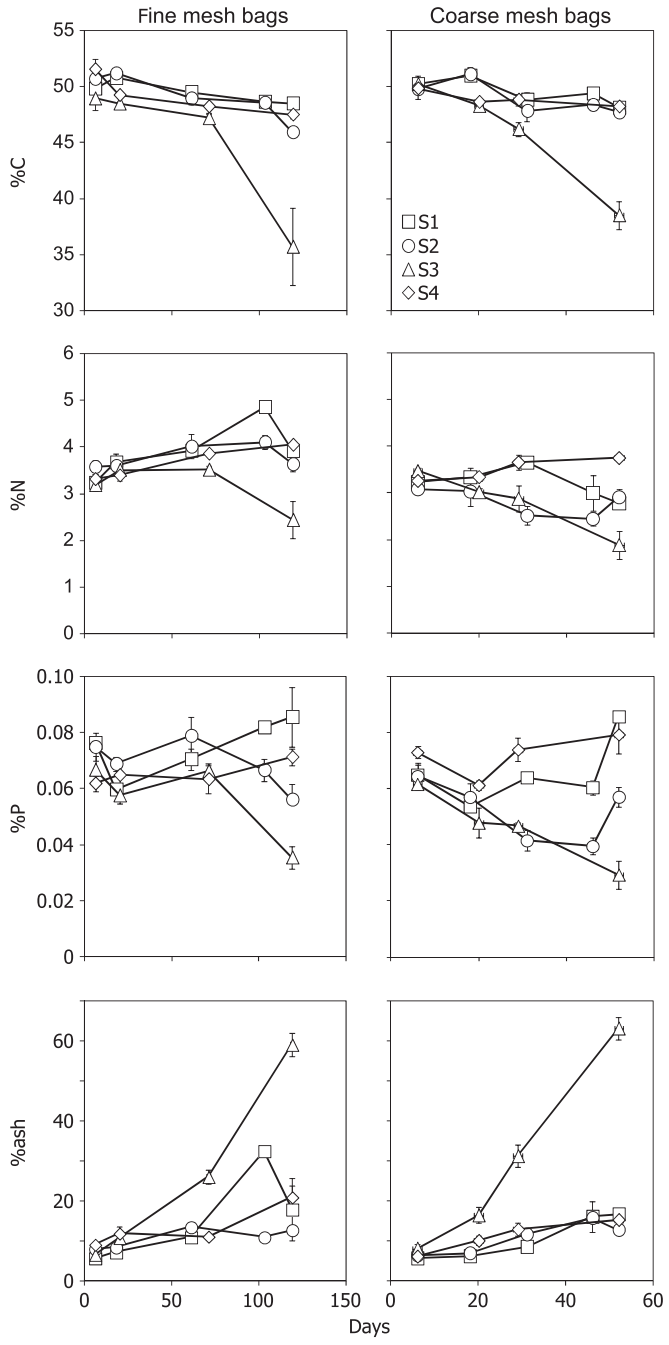

Fig. 2. Time course of variations in leaf carbon, nitrogen, phosphorous and ash concentration during decomposition process in fine (left) and coarse mesh bags (right). Streams are identified as follow: S1 square, S2 circle, S3 pyramid and S4 diamond.

The detritivore activity (estimated as the difference between the decomposition rate in coarse mesh and fine mesh bags) was related to shredder biomass associated with leaf-litter bags (Fig. 4; $r^2 = 0.93$, $p = 0.037$).

4. Discussion

The effects of flow intermittency on leaf-litter processing were more evident in coarse- than in fine-mesh bags. Although S3 and S4 suffered flow cessation just before the experiment start, S4 even dried up during the experiment, decomposition rates were quite similar among streams in fine mesh bags (i.e. mainly microbial activity). This lack of differences points out that microbial decomposer activity recovers quickly after rewetting. As Langhans and Tockner (2006) suggested, aquatic fungi activity can recover within one day after flow restoration, and our results agree with those reported by Bruder et al. (2011) in a temperate stream after experimental desiccation treatments. The resumption of microbial activity is possible because aquatic hyphomycetes can persist in moist substrata (Sanders and Webster, 1978; Sridhar and Bärlocher, 1993). It seems that the humid climate during winter in N-Spain can maintain enough moisture on a substrate such as leaf-litter even at terrestrial environments, as Langhans and Tockner (2006) pointed out in NE-Italy. This capability to survive in wet leaves permits microbial cells to rapidly colonize other substrates after flow restoration (Thomas et al., 1990; Dang et al., 2007). Moreover, the softer cuticle and low lignin content of alder, the species used in this work, facilitate the success of conidia settlement (Gessner and Chauvet, 1994; Kearns and Bärlocher, 2008; Dang et al., 2007). In addition, in the most intermittent stream, this survival capability in moisture substrates makes that microbial assemblages can follow alive during no-flow periods, resuming their activity following resubmergence. These results seem to be in contrast with those reported by Maamri et al. (2001) in an arid zone stream, where the likelihood of successful colonization of aquatic hyphomycetes decreased with the cessation of flow. This suggests, as Langhans and Tockner (2006) pointed out, that the effects of drought on microbial decomposer activity are related with duration of desiccation.

Contrary to the observed in fine bags, decomposition rates in the coarse ones differed among streams. However, we expected a greater effect of leaf-litter processing in relation to droughts, but only in the most intermittent of the two streams suffering dry periods the decomposition rate was diminished. Slower processing rates are often reported in temporary streams than those in the permanent ones (Herbst and Reice, 1982; Boulton, 1991; Pinna and Basset, 2004; Langhans and Tockner, 2006; Datry et al., 2011b) related with the reduction in density and richness of macroinvertebrates (Maamri et al., 1997; Acuña et al., 2005; Corti et al., 2011). These effects have been observed during dry events along the experiment but also long after post drought recovery (Schlief and Mutz, 2011; Martínez et al., 2013b), suggesting a "drying memory" as Datry et al. (2011b) stated. Therefore, we expected a similar response in the two temporary streams. In fact, benthic macroinvertebrate assemblages from S3 and S4 presented lower densities and biomasses than those in the other two permanent streams, being especially evident this difference in shredders. The communities from these temporary streams were dominated by pioneer taxa such as oligochaetes and dipterans, which present a typical community structure after rewetting (Stanley et al., 1994; Otermin et al., 2002; Acuña et al., 2005). Thus, the scarcity of principal consumers of leaf-litter helps to explain the slow breakdown rate in S4, where microbial activity was the major factor of decomposition since processing rates in both mesh bags were similar. This is also supported by the lack of differences in leaf nitrogen concentration between leaves incubated in fine and coarse bags in this stream. During in-stream decomposition, the leaf material often is enriched in nitrogen content as a consequence of microbial colonization, mainly fungi (Canhoto and Graça, 2008; Webster et al., 2009), since fungal mycelium shows higher %N than leaves (Cross et al., 2005). This renders the leaf material more palatable for detritivores (Graça and Cressa, 2010), which feed on most nutritive parts leading to a decrease in leaf-N to final stages of the process (Ferreira et al., 2006; Pérez et al., 2012). Thus, it was expectable that the %N of leaves from coarse bags (microbial activity and macroinvertebrate feeding) decreased along the decomposition.

Meanwhile, in S3, despite a poor benthic macroinvertebrate community, decomposition rate was similar to those in the permanent ones. This result seems to be related with the physicochemical characteristics of this stream, which may override the effects of droughts on leaf litter decomposition. S3 is characterized by travertine precipitation, resulting in a layer that covers the stream bottom and the standing substrates. In fact, leaves incubated in S3 presented an increment in ash content along the incubation period, as a consequence of the accumulation of $CaCO_3$

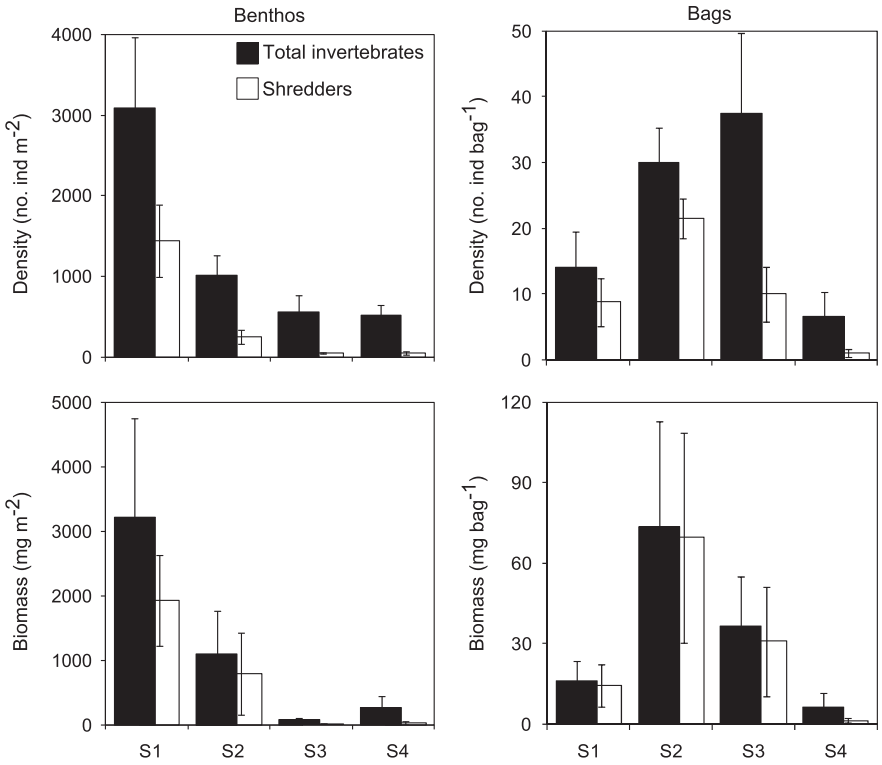

Fig. 3. Density (up) and biomass (bottom) of total macroinvertebrates (black bars) and shredders (white bars) in benthos (left) and in leaf-litter bags (right) after 29–31 days of incubation (*t*50).

(Casas and Gessner, 1999). This, as Casas and Gessner (1999) pointed out, may simulate siltation or burying of materials leading to delay leaf-litter processing. However, in our case, a different trend arises, being enhanced leaf-litter processing in spite of the lower density of principal benthic macroinvertebrate consumers as in karstic streams with a discontinuous travertine layer (Carter and Marks, 2007; Miliša et al., 2010). In the present study, the most suitable explanation is that the fauna find a food substrate in bags less affected by calcite precipitation, as the ratio of invertebrates in bags to invertebrates in benthos was highest in this stream. In addition, leaf species introduced by us was alder, which presents a better nutritional quality for consumers than *Q. faginea* (compare quality between *Alnus* and *Quercus* species in Ostrofsky, 1997), the dominant tree species in the surrounding forest. As often reported (Lecerf et al., 2005; Hladyz et al., 2009; Kominoski and Pringle, 2009), detritivores select actively high quality substrates to supply their stoichiometric demands (Sterner and Elser, 2002; Woodward, 2009). Thus, our experimental leaf packs would be acting as bait for macroinvertebrates in S3.

In summary, our results point out that the effects of drought events under oceanic climate conditions on leaf litter decomposition are more pronounced on invertebrate detritivore activity than on microbial decomposition. However, macroinvertebrate behavior imposed by the physicochemical properties of water, mainly travertine precipitation, can override the flow intermittence effects.

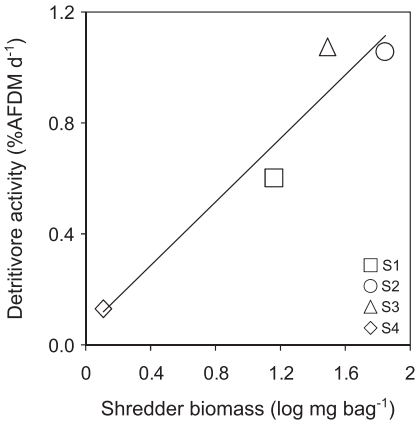

Fig. 4. Relationship between detritivore activity (breakdown rate in coarse bags — decomposition rate in fine bags, %AFDM d^{-1}) with shredder biomass (log mg bag^{-1}). Streams are identified as follow: S1 square, S2 circle, S3 pyramid and S4 diamond.

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